Influence of Rifampicin on the Pharmacokinetics of Salvianolic Acid B May Involve Inhibition of Organic Anion Transporting Polypeptide (Oatp) Mediated Influx

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This article studied the possible effect of rifampicin (RIF), an inhibitor of organic anion transporting polypeptide (Oatp), on the pharmacokinetics of salvianolic acid B (SAB) in rats. Rifampicin was administered intravenously 15 min prior to SAB (5 mg/kg) in rats at doses of 0, 5.0, 10.0 and 20.0 mg/kg, respectively. The concentrations of SAB in plasma and bile were determined using a Shimadzu HPLC system coupled to a LC-MS-2010EV mass spectrometer. Compared with the control group, the AUC₀₋₄ and Cmax values of SAB were increased significantly, while the CLtotal and CLbile were decreased significantly. These results suggested that pretreatment with rifampicin prior to SAB administration could decrease significantly the total and bile elimination of SAB and alter its pharmacokinetic profiles. The influence of rifampicin on the pharmacokinetics of SAB may be attributed to the inhibition of Oatp-mediated influx. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: salvianolic acid B (SAB); pharmacokinetics; organic anion transporting polypeptide (Oatp); rifampicin (RIF); drug–herb interaction.

INTRODUCTION
Cardiovascular disease seriously affects the expectancy and quality of people's life, accounting for 40% of mortality. Salvianolic acid B (SAB), the major water-soluble component isolated from the Salvia miltiorrhiza Bunge, has a wide spectrum of bioactivities, including protection of hepatocytes and hepatic stellate cells (Liu et al., 2002), antioxidants, antiatherosclerotic (Shiao et al., 2008) and inhibition of platelet aggregation (Li et al., 2004). Due to its important role in the treatment of cardiovascular disease, SAB has enormous potential for drug interactions in patients who often receive other drugs. For instance, clinically important pharmacokinetic and pharmacodynamic interactions between Salvia miltiorrhiza extract and warfarin were observed when these two agents were taken together (Chan, 2001).
It is well known that carrier-mediated transport contributes to hepatic uptake and/or biliary excretion for many endogenous and exogenous compounds. According to our previous study in our laboratory, only a small fraction of the injected dose was excreted from urine after i.v. injection, but more from bile relatively (Ma et al., 2007). It is rapidly and readily excreted into the bile, leading to a higher drug concentration in bile than that in blood. The distribution ratio of AUCbile/AUCblood of SAB suggests that the hepatobiliary elimination of SAB may be regulated by an active transporter (Chen et al., 2005). Rifampicin is used clinically in the treatment of tuberculosis. It may need a long-term combination therapy for an accompanying disease (Mahatthanatrakul et al., 2007). However, the effect of rifampicin on liver is very complex. It is used broadly in clinical studies as a representative inducer of drug-metabolizing enzymes and transporters (Tirona et al., 2003). In addition, it has been shown to be an excellent substrate and inhibitor of organic anion transporting polypeptides (OATPs in human/Oatps in rats) (Lam et al., 2006). Recently, it has been reported that many significant drug interactions are associated with rifampicin (Mallolas et al., 2007; Ribera et al., 2007).
Liver is vital in the disposition of rifampicin and SAB, so potential drug interaction between them is possible through competition for the transporters. Given the current lack of published data regarding the drug–herb interaction between rifampicin and SAB, the aim of this study was to investigate the possible effect of rifampicin on the pharmacokinetics of SAB in rats.

MATERIALS AND METHODS

Materials. Salvianolic acid B, isolated from the roots of Salvia miltiorrhiza, with a purity of over 98% by HPLC analysis, was obtained from the Center of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University (Nanjing, China). Genistein and rifampicin were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile of HPLC grade was from Tedia (USA). All other reagents were of analytical grade.

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**Animals.** Male Sprague–Dawley rats (200 ± 20 g) were provided by the Shanghai Sino-British Sippr/BK LAB Animal Co. Ltd (Shanghai, China). Animals were housed under controlled conditions (20 ± 2 °C, H 50 ± 20%) with a natural light–dark cycle, allowed to adapt to the housing environment for 1 week prior to study and were fasted overnight (12 h) with free access to water throughout the experimental period. The studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

**Experimental design.** The rats were divided into four groups, five rats per group. Before surgery, the rats were anesthetized by intraperitoneal injection with urethane (20%; 1 g/kg). Then the bile duct was isolated and cannulated with a polyethylene (PE) tube (0.4 mm, i.d. and 0.8 mm, o.d.; Natsume, Tokyo, Japan). Rifampicin was administered intravenously from tail to groups II–IV at a dose of 5.0, 10.0 and 20.0 mg/kg, respectively. After 15 min, SAB was injected via the tail vein (5 mg/kg). The same procedure was carried out in group I without rifampicin. Blood samples were collected in heparinized Eppendorf tubes at 0, 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240 min after administration and centrifuged immediately at 12000 × g for 3 min. Plasma of 100 µL volume was finally harvested. Bile samples were collected at 0–0.25, 0.25–0.5, 0.5–1, 1–2 h into tubes and the volume of each sample was recorded. All samples were stored at –20 °C until analysis.

**Sample preparation and the LC-MS assay.** Plasma and bile samples were analysed by a Shimadzu HPLC system coupled to a LC-MS-2010EV mass spectrometer (Shimadzu, Japan). The process was described as follows. Briefly, a fresh sample (100 µL) was spiked with 10 µL of HCl (2.5 mM). Then 10 µL of genistein (10 µg/mL, in methanol) was added as an internal standard. The mixture was extracted with 500 µL of ethyl acetate by vortex-mixing for 3 min. Following centrifugation (12000 × g, 5 min), 400 µL of organic layer was evaporated to dryness under a stream of air at 37 °C. The residue was dissolved in 100 µL of the mobile phase and 20 µL of the sample was injected into the LC–MS system. The analytical column was a Hypersil C18 column (5 µm, 4.6 × 200 mm i.d., Elite Co., Dalian, China) kept at 28 °C in the CTO-20A column oven. The mobile phase consisted of acetonitrile–water containing 0.75% formic acid (45:55, v/v) at a flow rate of 1.0 mL/min. The HPLC/ESI/MS analysis was performed in negative ion selected ion monitoring (SIM) mode using target ions at m/z 717 for SAB and m/z 269 for the internal standard (IS) genistein. A quadrupole mass spectrometer equipped with an electrospray ionization source was operated with a drying gas (N2) flow of 1.5 L/min, the detector voltage at 1.50 kV and probe voltage at 3.5 kV. The heat block temperature was 250 °C and the curved desolvation line (CDL) temperature was maintained at 230 °C. The mobile phase was degassed using a DGU-20A3 degasser and mixed with a CBM-20A rotary pump. Samples were introduced using a SIL-20 AC auto-injector with an effective volume of 20 µL. Data acquisition and processing were accomplished using Shimadzu LC-MS solution (Version 3.20 with Windows XP operating system).

The calibration curve of SAB was linear within the range 0.05–50 µg/mL in both plasma and bile samples (R² > 0.999). The relative standard deviation (RSD) of inter- and intra-day precisions were both less than 10%. The relative recovery of SAB from plasma and bile samples was more than 60%.

**Pharmacokinetic analysis.** The pharmacokinetic parameters were calculated using the pharmacokinetic software WinNonlin Professional Version 5.0.1 (Pharsight Corp., Mountain View, CA, USA) by a non-compartmental method. The peak plasma concentration (Cmax) was directly obtained from the observed concentration–time data. The area under the plasma concentration–time curve (AUC) from time zero to the last measured concentration (AUC0–t) was calculated according to the linear trapezoidal rule. The terminal elimination rate constant (γ) was estimated by linear regression of the terminal portion of the Ln (concentration)–time curve, and the elimination half-life (t1/2) was calculated as 0.693/γ accordingly. Bile clearance (CLbile) was calculated as CLbile = Ae × AUC, where Ae (amount of unchanged drug eliminated in bile) and AUC were measured over the same time interval.

**Statistical analysis.** All data are presented as mean with the standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, using the Dunnett correction. The p value for statistical significance was set at <0.01.

**RESULTS**

The mean plasma concentration–time curves of SAB after intravenous administration at a dose of 5 mg/kg to rats in the presence and absence of rifampicin are shown in Fig. 1. The mean biliary excretion–time profiles under the same condition are shown in Fig. 2. As shown in Fig. 1 and Fig. 2, the concentrations in plasma and bile of SAB were significantly changed by pretreatment with rifampicin. The mean pharmacokinetic parameters were summarized in Table 1. Compared with the control group (group I), the AUCt,0 was significantly greater (161.3%, 315.9% and 597.9% increase for groups II–IV, respectively) with

![Figure 1. Mean plasma concentration–time curves of salvianolic acid B (SAB) in rat plasma after intravenous administration at a dose of 5 mg/kg to rats in the presence and absence of rifampicin (RIF) (mean ± SD, n = 5 per group). □, control; △, rifampin, 5 mg/kg; ▼, rifampin, 10 mg/kg; X, rifampin, 20 mg/kg. Bars represent standard deviations.](image-url)
rifampicin, while the CL_{bile} was significantly slower (84.2%, 92.9% and 99.1% decrease for groups II–IV, respectively) after intravenous administration of SAB.

In addition, no significant changes in the SAB terminal half-life (t_{1/2}) were observed between intravenous dosing with and without rifampicin. It seems that rifampicin does not affect the t_{1/2} of SAB. After calculation, data (not shown) indicated that pretreatment with rifampicin increased plasma levels and decreased biliary excretion of SAB in a dose-dependent manner.

**DISCUSSION**

The present study examined animal models that may predict the interaction between rifampicin and SAB. The results showed that pretreatment with rifampicin could significantly alter the bile elimination and pharmacokinetic profiles of SAB and the changes were consistent with the dosage of rifampicin. However, no significant changes in the SAB terminal half-life (t_{1/2}) were observed between intravenous administration with and without rifampicin. It appears that rifampicin does not affect t_{1/2}. It is also reported that the t_{1/2} of atorvastatin and its hydroxy metabolites were not affected by rifampicin or the route of administration, which is similar to our situation (Lau et al., 2006).

The effects of rifampicin on drug metabolism and transport are broad and significant. The molecular mechanisms of the interactions of rifampicin with other drugs have not been fully understood until recently. On the whole, they can be divided into three types. Firstly, rifampicin is a potent inducer of drug metabolizing enzymes. It strongly induces the expression of CYP450 3A4 both in liver and intestine (Yasuda et al., 2008). Other enzymes are induced as well, such as CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 3A5, monoamine oxidase-B (MAO-B), flavin containing mono-oxygenases 4 and 5 (FMO4, FMO5), UDP-glucuronosyltransferases (UGTs) and so on (Niemi et al., 2003). Secondly, P-glycoprotein (P-gp) (Greiner et al., 1999), multiple drug resistance protein 2 (mdr2) (Fromm et al., 2000), Oatp2 (Staudinger et al., 2001) and other transporters were induced in the same manner as for enzymes. In this situation, the concentration of drugs that were substrates for these enzymes and/or transporters would be greatly reduced and their oral bioavailability limited, possibly resulting in therapeutic failure. Thirdly, Oatp was inhibited and thereby the plasma concentration of substrate would be increased significantly.

Full induction of drug-metabolizing enzymes is reached in about 1 week after starting rifampicin treatment and the induction dissipates in roughly 2 weeks after discontinuing rifampicin (Yasuda et al., 2008), while the inhibition occurred faster. In our study, after pretreatment with a single dose of rifampicin prior to SAB administration, the AUC and C_{max} values of SAB were significantly increased, while the CL_{total} and CL_{bile} values were significantly decreased. Thus, it might be speculated that rifampicin would inhibit Oatp1b2 and the inhibition would decrease the hepatic uptake of SAB and possibly weaken excretion into the bile.

Recent years have witnessed a rapid development of methods investigating the important role of OATP/Oatp. The Oatp1b2 knockout mouse model has been generated which is suitable for understanding the in vivo role of hepatic Oatp transporters in drug disposition (Zaher et al., 2008; Chen et al., 2008). Rifampicin has been shown to be an excellent inhibitor of Oatp1b2 localized on the sinusoidal membrane of hepatocytes. In a transiently transfected cell line (Chinese hamster ovary), rifampicin significantly inhibited OATP1B1-mediated uptake of bosentan (Treiber et al., 2007). In an isolated perfused rat liver system, rifampicin reduced the AUC of digoxin significantly without affecting enzymatic activities (Lau et al., 2004). In vivo, after rifampicin administration, maximum serum concentrations of

![Figure 2. Mean biliary excretion-time profiles of salvianolic acid B (SAB) after intravenous administration at a dose of 5 mg/kg to rats in the presence and absence of rifampicin (RIF) (mean ± SD, n = 5 per group). **control; □, rifampin, 5 mg/kg; ×, rifampin, 10 mg/kg; ◯, rifampin, 20 mg/kg. Bars represent standard deviations.](image)

**Table 1.** Mean pharmacokinetic parameters of salvianolic acid B (SAB) in rat plasma after intravenous administration at a dose of 5 mg/kg to rats in the presence and absence of rifampicin (RIF) (mean ± SD, n=5)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SAB alone (5 mg/kg)</th>
<th>SAB + RIF (5 mg/kg)</th>
<th>SAB + RIF (10 mg/kg)</th>
<th>SAB + RIF (20 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_{1/2} (min)</td>
<td>107.3±49.2</td>
<td>71.4±24.8</td>
<td>74.1±13.9</td>
<td>93.2±20.7</td>
</tr>
<tr>
<td>C_{max} (µg/mL)</td>
<td>17.9±3.9</td>
<td>33.9±1.9*</td>
<td>38.5±6.3*</td>
<td>47.6±6.1*</td>
</tr>
<tr>
<td>AUC0-t (µg·min/mL)</td>
<td>200.6±56.7</td>
<td>524.3±83.9*</td>
<td>834.5±224.8*</td>
<td>1400.4±134.8*</td>
</tr>
<tr>
<td>AUC0-∞ (µg·min/mL)</td>
<td>213.3±58.3</td>
<td>564.4±120.2*</td>
<td>907.4±246.2*</td>
<td>1610.7±162.0*</td>
</tr>
<tr>
<td>CL_{total} (mL/min/kg)</td>
<td>24.9±6.9</td>
<td>9.2±1.8*</td>
<td>5.8±1.5*</td>
<td>3.1±0.3*</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>30.0±6.7</td>
<td>47.4±8.6*</td>
<td>52.2±2.7*</td>
<td>64.8±3.1*</td>
</tr>
<tr>
<td>Ae (%dose)</td>
<td>36.2±6.7</td>
<td>14.6±2.4*</td>
<td>10.2±2.1*</td>
<td>2.2±0.4*</td>
</tr>
<tr>
<td>CL_{bile} (mL/min/kg)</td>
<td>2.099±0.809</td>
<td>0.331±0.091*</td>
<td>0.149±0.043*</td>
<td>0.019±0.003*</td>
</tr>
</tbody>
</table>

*< 0.01, significant difference compared with the control (given SAB alone).
etzetimibe and its glucuronide were apparently elevated (Oswald et al., 2006).

A previous study indicated that relatively large (molecular weight > 400–500 kDa) and hydrophobic organic anions, such as bile salts and pravastatin, are preferentially excreted via the liver (Sekine et al., 2006). They are preferable substrates of members of the Oatp family. SAB has a carboxyl group and phenolic hydroxyls and a molecular weight of 718.6, and exists in the ionic form in alkaline plasma. In short, SAB is consistent with the transporter substrate characteristics of the Oatp family.

In conclusion, this is the first study investigating the mechanisms of the potential pharmacokinetic interactions between SAB and rifampicin in vivo. The results indicate that the influence of rifampicin on the pharmacokinetics of SAB may involve inhibition of Oatp-mediated influx.

Acknowledgements

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Conflict of Interest

The authors have declared that there is no conflict of interest.